4-11-2017

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Published in *Journal of Comparative Physiology A* 203 (2017), pp 313–328. DOI 10.1007/s00359-017-1169-5

Submitted 9 August 2016; revised 2 February 2017; accepted 30 March 2017; published online 11 April 2017.

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Abstract
Amblypygids are capable of navigation in the complex terrain of rainforests in near complete darkness. Path integration is unnecessary for successful homing, and the alternative mechanisms by which they navigate have yet to be elucidated. Here, our aims were to determine whether the amblypygid *Phrynus marginemaculatus* could be trained to reliably return to a target shelter in a laboratory arena—indicating goal recognition—and to document changes in behavior associated with the development of fidelity. We recorded nocturnal movements and space use by individuals over five nights in an arena in which subjects were provided with two shelters that differed in quality. The target shelter, unlike the alternative shelter, shielded subjects from light in daylight hours. Individuals consistently exited and returned to a shelter each night and from the third night onward chose the target shelter more often than the alternative shelter. Indeed, on the fifth night, every subject chose the target shelter. This transition was associated with changes in movement and space use in the arena. Notably, the movement features of outbound and inbound paths differed but did not change across nights. Individuals were also characterized by distinct behavioral strategies reflecting candidate homing mechanisms.

Keywords: Amblypygid, Arachnid, Navigation, Nocturnal, Homing

Introduction

Navigation in arthropods is supported by a variety of behavioral mechanisms (Papi 1992; Gould 1998; Dyer 1998; Collett and Graham 2004; Collett and Collett 2006; Collett et al. 2013; Cheng 2012; Cheng and Freas 2015; Knaden and Graham 2016), and the navigational behavior of diurnal, visually-oriented species is particularly well documented (Perry et al. 2013). The majority of these study species inhabit generally uncluttered environments in which information from one or more sensory modalities combined with path integration is sufficient to locate a goal. In contrast, relationships among the behavioral–sensory mechanisms that underlie navigation and orientation in environments that impose difficult spatial, structural, and informational challenges are comparatively unexplored (Warrant and Dacke 2010, 2011, 2016; Jeffery et al. 2013; Baird and Dacke 2016).

In a recent paper, we proposed that amblypygids, also known as whip spiders or tailless whip scorpions, could serve as potential model organisms for the study of arthropod navigation in complex environments. These unusual arachnids are distributed worldwide in the tropics and subtropics (reviewed by Weygoldt 2000; Harvey 2007; Chapin and Hebets 2016). The majority of amblypygid species inhabit rainforests, where, in daylight hours, they hide in hollow trees, under tree bark or in the crevices of tree buttresses. They emerge after dusk to forage and return to their shelter before

dawn. Unlike spiders, amblypygids walk on six legs. Their anterior pair of legs—no longer used for locomotion—are called antenniform legs. These legs are highly articulated and covered with thousands of mechanosensory and chemosensory sensilla (Foelix et al. 1975; Beck et al. 1977; Santer and Hebets 2011). Field studies of *Heterophrynus* and *Phrynus* species revealed that individuals often wander but typically reside in the same location for weeks or months (Beck and Görke 1974; Weygoldt 1977; Hebets 2002).

Beck and Görke (1974) were the first to document the navigational abilities of amblypygids. These researchers captured nine *Heterophrynus batesii* (a large Amazonian species) when they emerged at night from tree crevices and displaced them distances of 2.5–7.5 m, where they were placed on the ground. Every individual returned to the tree on which it was captured on the same night that it was displaced. Beck and Görke (1974) also displaced one subject 10 m and observed that it too returned sometime between 2 and 5 nights later.

More recent nocturnal displacement experiments conducted with two Central American species of amblypygid revealed similar navigational abilities (Hebets et al. 2014a; Bingman et al. 2017). *Paraphrynus laevifrons* (misidentified as *Phrynus pseudoparvulus* in Hebets et al. 2014a) were displaced distances of 6–9 m and tracked with radio transmitters. Subjects typically returned in a single night to the tree from which they were captured. Additionally, experiments in which *P. pseudoparvulus* were displaced to trees from which residents were removed showed that particular trees do not act as attractor beacons (Hebets et al. 2014a). Members of these species are also capable of navigating from more challenging displacement distances, at least as far as 25 m, but these journeys usually involve a temporary residency at another tree or in a burrow (Hebets 2002). These field experiments revealed that path integration, a navigation strategy used by all studied terrestrial arthropods, is not necessary for successful navigation by amblypygids. How these animals navigate in the darkness of a nocturnal rainforest and come to recognize their particular tree remains a biological mystery.

In this study, we used the species *Phrynus marginemaculatus*, which is native to southern Florida (USA), the Bahamas, and surrounding Caribbean islands (Quintero 1981; Weygoldt 2000; Chapin and Hebets 2016). This species is strictly nocturnal and inhabits subtropical rainforests such as South Florida rockland and Bahamanian pine forests (Quintero 1981; Weygoldt 2000), which can be similar in structural complexity to tropical rainforests (United States Fish and Wildlife Service 1999). Thus, we expect that these habitats could impose navigational challenges that are comparable to those experienced by previously studied, tropical species of amblypygid, although some populations of *P. marginemaculatus* do inhabit structurally simpler environments. In this paper, our goals were to establish that *P. marginemaculatus* can be trained to show shelter-recognition behavior in the laboratory that mirrors—on a smaller scale—the navigation behavior of amblypygids in the field, and more importantly, to describe the development of shelter fidelity and characterize changes in movement and space use as fidelity to a shelter develops.

Materials and methods

Experimental animals

In this study, we used a total of 12 adult *P. marginemaculatus*. We purchased seven wild-caught individuals from a commercial supplier (Ken The Bug Guy, LLC). The other five individuals were collected at the National Key Deer Refuge (Big Pine Key, Florida, USA; USFWS Permit Number FFO4RFKD-2015-06). In our laboratory, individuals were housed separately in circular plastic deli cups (diameter × *H*: 17.1 cm × 10.8 cm) that had a soil substrate and a piece of cardboard egg carton for shelter.

Animals were fed live crickets and misted with reverse osmosis water three times per week. The room in which animals were housed was lit with overhead broad-spectrum fluorescent lights (400–750 nm) set to a 12:12 h light:dark cycle (15:00–03:00 laboratory dusk–dawn). We kept animals on this light cycle for several weeks before the study was conducted. The room temperature ranged from 21 to 26 °C and humidity ranged from 20 to 60%.

Experimental design and apparatus

Each subject was placed individually into one of two arenas. Each arena contained two shelters that differed in their level of protection from light in daytime hours and an odor source that was positioned near the better-shielded shelter. It is important to note that the odor source was not used to test a hypothesis of olfactory navigation. There is good evidence that odors are used to assist navigation (Hansson and Stensmyr 2011; Jacobs 2012; Svensson et al. 2014), so we included the odor source to increase environmental heterogeneity and facilitate the spatial discrimination of the two shelters. We monitored activity and space use over a session of five nights with an automated, real-time video tracker that continually recorded the coordinates of a subject when it was outside of a shelter. Subjects were fed the day before and immediately after the experimental session, but were not fed during the experimental session.

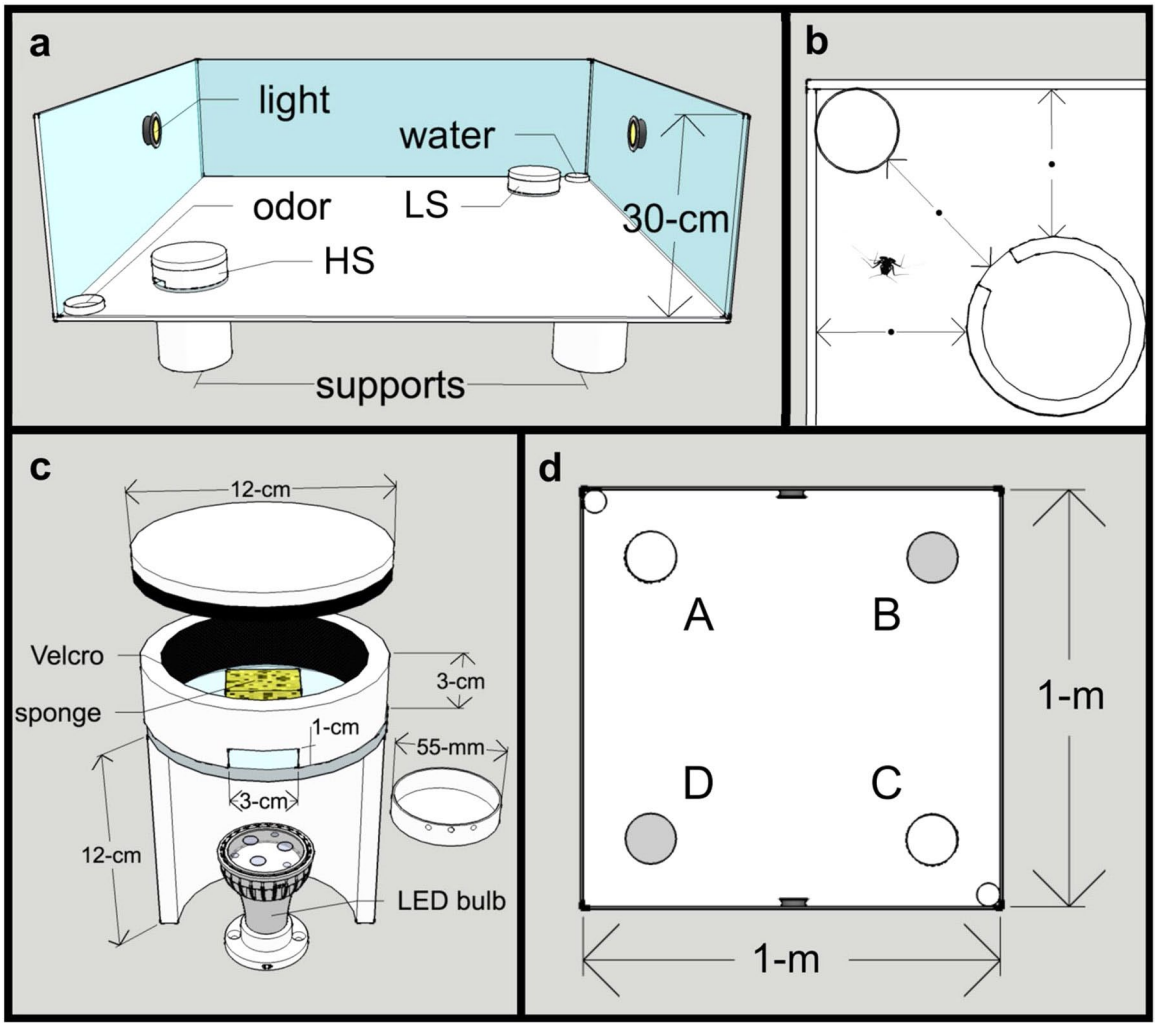


Fig. 1. The experimental setup. **a)** Layout of the arena. **b)** A detailed view of the placement of the shelter and odor source in relation to the size of the animal. **c)** A detailed view of the shelter, a corner support (under shelter), and the odor source (small dish on *right*). LED output was covered with either clear plastic (LS) or opaque, black plastic (HS). *Arrow* spans indicate a distance of 10 cm. **d)** A top-down view of the arena with possible shelter locations (*gray* and *white circles*) indicated by *A–D* and possible combinations (*A, C* or *B, D*) indicated by *gray* and *white shading* in the *circles*.

The arenas

Figure 1 shows a schematic of the arena design ($L \times W \times H$: 1 × 1 × 0.3 m). The bottom of each arena was constructed from a single piece of opaque white acrylic plastic, which created visual contrast between a subject and the arena floor to enhance the detection of a subject by the video tracker. The walls were built of clear acrylic plastic. Two 5-W, broad-spectrum halogen lights were attached to two of the walls to motivate subjects to use a shelter during daylight hours. Each arena was elevated 12 cm off the floor by four support posts made of 12-cm (outer diameter) PVC pipe placed underneath the arena near each corner.

The two shelters were constructed from 12-cm (outer diameter) PVC pipe cut to a height of 3 cm. The top of each shelter was fitted with a circular piece of black opaque acrylic plastic (to block light) that was covered by a circular piece of white opaque plastic (to facilitate detection of a subject by the video tracker if it walked on the top of the shelter). The bottom of the shelter was fitted with a circular piece of transparent acrylic plastic. Each shelter had an entrance ($L \times H$: 3 × 1 cm) located at the level of the arena floor. The interior walls of the shelters were lined with black Velcro ® tape (hook and loop fastener fabric with an adhesive surface) for subjects to climb on, and each shelter contained a cellulose sponge saturated with reverse osmosis water for humidity. The shelters were positioned in opposite (diagonal) corners of an arena, directly over the support posts, with each shelter entrance directed toward the nearest corner. Shelters and supports for

each arena were placed 10 cm from the nearest wall, such that the shelter entrance was located approximately 16 cm from the nearest corner (Fig. 1b).

The PVC supports under each shelter contained a 3-W, broad-spectrum, high-power LED light pointed upward toward the transparent bottom of a shelter, and this light was turned on in daylight hours. The floor of the arena was sufficiently transparent for light to pass into the shelters, and whether we allowed the LED light to be transmitted into a shelter determined its presumed quality. The light positioned under the low-quality shelter (LS) was covered with a piece of 95% transparent acrylic plastic. The light under the high-quality shelter (HS) was covered with a piece of opaque black acrylic plastic that blocked the LED light and kept the shelter dark in daylight hours.

Olfaction, mediated by receptors located on the distal segments of the antenniform legs, is hypothesized to facilitate navigation by amblypygids (reviewed by Chapin and Hebets 2016). As noted above, in this experiment, we provided an olfactory cue near the HS in the form of geraniol to enhance the spatial heterogeneity of the experimental environment and promote shelter discrimination but not to test whether the odor was used for homing (although it was likely it would be). Geraniol is a component of many plant-based essential oils and was chosen as an odor source because it is detected by numerous terrestrial arthropods (Hansson and Stensmyr 2011; Leonard and Masek 2014). Because amblypygids do not rely directly on plants as a food source, we hypothesized that geraniol would be neither a particularly attractive nor aversive stimulus. Furthermore, *P. pseudoparvulus* is known to detect monoterpenoids that are similar in molecular structure to geraniol (Hebets and Chapman 2000). The source for dispersal of the odor was a 55 × 16 mm (diameter × *H*) plastic petri dish into which we inserted a 50-mm diameter piece of circular filter paper (Whatman® qualitative filter paper, Grade 1) laden with 15 μL of geraniol (Sigma-Aldrich, Product Number 163333). The petri dish had three 3-mm holes spaced 1 cm apart drilled into its side that allowed the odor to disperse into the air and restricted subjects from direct contact with the filter paper. The odor was not replenished within an experimental session but was still detectable by us when a session ended.

Lighting and camera

The experiment was conducted in the same room that the animals were housed. The halogen lights attached to the arena walls and LED lights placed under the shelters were kept on a timer that turned them off 1 h before the overhead lights and turned them on 1 h after the overhead lights were turned on (thus, the overhead lights determined laboratory night and day). The room was kept completely dark at night except for two 9-W red-filtered compact fluorescent lamps (610–700-nm) and high-power infrared LED flood lights (~850 to nm) that provided illumination to an infrared sensitive camera (Avemia Vari-focal CCTV Camera CMBB100) mounted above each arena. Each camera was connected to an analog-to-digital converter box (Canopus ADVC-110) which was connected to a PC that ran custom-written software used to track animals. Both of these lamps produce a light spectrum outside the range (300–600-nm) to which *P. marginemaculatus* are sensitive (Graving et al. in prep.).

Video tracker

The Cartesian coordinates of a subject in the horizontal space of the arena were extracted from each video frame by our own custom-written software POSE (Graving 2016) or software written using functions from the JavaGrinders library, a collection of freeware programming functions for automated analyses of behavioral data (available at http://iEthology.com). Our custom software was written in Python 2.7.11 (Python Software Foundation, available at http://www.python.org) and utilized OpenCV 2.4.11 (Pulli et al. 2012). The software used a Gaussian mixture-based foreground–background segmentation algorithm (as implemented in cv2.BackgroundSubtractorMOG) to separate each video frame into the foreground (subject) and background (arena floor) and then fitted a contour to the outline of the segmented image of the subject (using cv2. contours). The Cartesian coordinates of the centroid of an animal were extracted using the image moments (calculated by cv2. moments). As a subject moved around the arena, timestamps and coordinates of the animal centroid were recorded once every 2 s and were saved to a text file that was later used to calculate spatial statistics and kinematic variables. The tracker was calibrated to record the movements of subjects in the arena at night and in the 1-h transitory period between day and night when the lights on arena walls were off and the overhead lights were on but did not record subject positions once the lights on the arena walls were activated.

Procedures

Several hours before the start of a session, the shelters and odor dishes were cleaned with an unscented, aqueous detergent solution, rinsed with hot tap water and allowed to air dry. The arena floor and walls were wiped with 95% ethanol and allowed to air dry until the ethanol odor dissipated. The position of the HS for each of the 12 subjects was chosen from one of four fixed positions, designated A, B, C, and D, directly above

the four arena supports in a balanced, randomized design such that the position of the HS was positioned in each location for exactly three of the total 12 subjects/sessions, and the LS always positioned in the opposite (diagonal) corner (Fig. 1d).

Approximately 3 h before laboratory night, a randomly selected subject was removed by hand from its home container and placed into a clean plastic container for several minutes until its movement slowed and it no longer appeared startled. The animal was then placed directly inside the HS by removing the HS lid, slowly inverting the plastic cup until the animal slid inside the shelter, and replacing the HS lid. A petri dish with geraniol was placed in the adjacent corner with the perforated side of the dish directed toward the center of the arena, approximately 10 cm from the entrance to the shelter. In the corner adjacent to the LS we placed a petri dish that was prepared with 15 μL of reverse osmosis water. We then calibrated the camera to nighttime conditions and started the tracker. The start of a five-night session began when the overhead lights subsequently turned off.

Each morning, we examined the tracking data, visually verified shelter choice, and restarted the tracker. If a subject failed to exit a shelter on a particular night, the session was extended for an extra night and continued until a subject exited a shelter on five—not necessarily consecutive— nights. Ten of the 12 subjects completed the session in five consecutive nights, and eight of these ten subjects were held in the arena for an additional sixth night to determine whether shelter fidelity continued. For formal analyses, however, we used only data from the first five nights on which a subject exited and returned to a shelter.

Variables

Trajectory data were plotted and checked visually for errors. Erroneous coordinates were removed. We rescaled coordinates from pixel units into real distance units, and for analytical and visualization purposes, we rotated the coordinate space so that the HS and LS were in the same location for all subjects. We then spatially rediscretized the data following procedures described by Bovet and Benhamou (1988). In brief, we resampled each trajectory as a series of movement vectors, or steps, such that the magnitude, or step length, of each vector was ~2.5 cm. Importantly, this re-discretization procedure removes movement artifacts produced by the tracking algorithm and ensures that the distributions of kinematic variables derived from these trajectories are unbiased. The variables that we scored from trajectory data and their random expectations, where appropriate, are summarized in Table 1.

Analyses

Generalized linear mixed models (GLMM) were fitted separately to the variables in Table 1 to characterize the development of shelter fidelity, phenology, space utilization, and nightly outbound and inbound paths. In all models, intercepts and slopes were coded as random effects, by subject—nested, where appropriate—to account for the repeated measurements on each of the subjects (Bolker et al. 2009). The fixed effect(s) included in a model are described for each specific analysis.

Shelter fidelity

The shelter choice of subjects at the end of each night was determined from the video records, visually verified from the location of an animal. We fitted a GLMM that included shelter choice as the response and night number as a fixed effect to determine the rate at which shelter fidelity developed.

Phenology and general activity

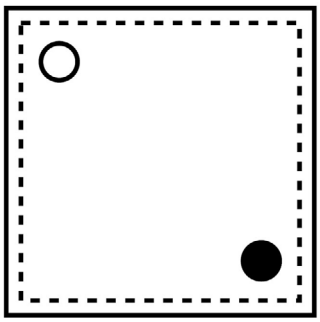
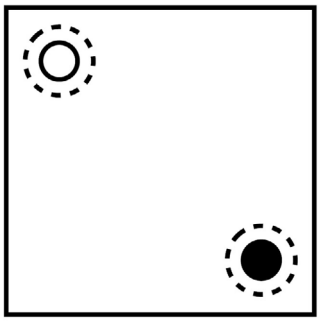
We recorded several variables to characterize phenology and general activity. These included when a subjected exited and entered a shelter, the total time an animal was outside a shelter and the total distance that a subject traveled each of the five nights (Table 1). We fitted a GLMM to each variable with night number as a fixed effect to determine how the timing of activity and movement of the subjects changed across nights. We excluded one observation as an outlier in which a subject entering a shelter occurred outside of the 12-h dark period.

Space utilization

The first measure of space utilization that we recorded was wall following. In visually deprived species, wall following behavior often decreases with time after introduction to a novel environment, and this pattern is attributed to memorization of the spatial environment (reviewed by Patton et al. 2010). Amblypygids often use their antenniform legs to guide their movement (Santer and Hebets 2009), so we defined wall following as the proportion of steps less than or equal to the maximum length of the antenniform legs, or 5 cm, from a wall. We also measured proximity to the HS and LS, defined for the same reason as the proportion of steps in a night that a subject was within 5 cm of a shelter wall.

We fitted a GLMM to each of these variables to determine how they changed across nights. For wall following, we included night number as a fixed effect and for shelter proximity, we included night number, shelter quality, and the interaction between night number and shelter quality as fixed effects.

Table 1. Descriptions of measured variables, separated by analyses

Analysis	Variable	Description	Illustration
Site fidelity	Shelter choice	Binary choice of shelter (HS = 1, LS = 0) for each subject on each night on which a subject was active in the arena	
Phenology and activity	Exit time (h)	The time interval between lights off and when a subject exited the shelter	
	Enter time (h)	The time interval between when the animal returned to a shelter and lights on	
	Activity time (h)	The time interval between when an animal exited and entered a shelter	
	Total distance (m)	The total distance moved by a subject over the course of a night	
Space utilization	Wall following	Proportion of steps within 5 cm distance of a wall. The random expectation that an animal is found in this area is 0.19, the outer 5 cm area divided by the total area	
	Shelter proximity	Proportion of steps within 5 cm distance of a shelter. The random expectation that an animal is found in this area around each shelter is 0.027, the area circumscribed around a shelter divided by the total area	
Path kinematics	Mean speed (mm s^{-1})	Averaged linear speed (distance $time^{-1}$) of the steps in a trajectory	
	Mean distance to wall (cm)	Averaged distance between each step and the nearest wall in a trajectory	
	Sinuosity (rad $\sqrt{m}-1$)	$S = 1.18 \frac{\sigma_R}{\sqrt{R}}$ where R is the rediscretized step length of the trajectory, σ_R is the angular deviation of the distribution of relative step angles, or changes in direction, in a trajectory with step length R, and R is selected such that $0.1 \leq \sigma_R \leq 1.2$	See Bovet and Benhamou (1988) for details
	Mean path vector	The unit mean vector of steps within a trajectory from the end point (outbound) or start point (inbound) of the trajectory to the entrance of the HS	

Illustrations show how various variables were calculated, where filled and open circles represent the HS and LS, respectively

Outbound and inbound paths

The termination of an outbound path was defined by the location of a subject where it first exceeded 0.84 m from the entrance of the shelter. The start of the inbound path was likewise defined as the last point of a night where a subject was farther than 0.84 m from the entrance of the shelter. The distance between the HS entrance and the

Table 2. GLMM results

Response	Family	Link	Transform	Fixed effect	Estimate	SE	*z*	$P > \|z\|$	[2.5%, 97.5%]
Shelter choice (HS = 1, LS = 0)	Bernoulli	Logit	–	(Intercept)	0.859	0.626	1.372	0.170	[0.299, 5.610]
				Night	**0.697**	**0.299**	**2.331**	**0.020**	**[0.232, 2.688]**
Exit time (h)	Gamma	Log	power (λ = 0.25)	(Intercept)	−0.311	0.335	−0.930	0.345	[−2.304, 1.216]
				Night	0.034	0.045	0.772	0.431	[−0.410, 0.417]
Enter time (h)	Gaussian	Identity	Box–Cox (λ = 0.5)	(Intercept)	0.444	0.386	1.149	0.246	[−0.313, 1.201]
				Night	**0.259**	**0.081**	**3.188**	**0.001**	**[0.100, 0.417]**
Activity time (h)	Gaussian	Identity	–	**(Intercept)**	**9.040**	**0.963**	**9.390**	**<0.001**	**[7.222, 10.954]**
				Night	**−0.513**	**0.221**	**−2.318**	**0.020**	**[−0.951, −0.091]**
Total distance (m)	Gaussian	Identity	Box–Cox (λ = 0.5)	**(Intercept)**	**25.280**	**2.763**	**9.151**	**<0.001**	**[19.865, 30.694]**
				Night	**−2.461**	**0.717**	**−3.431**	**<0.001**	**[−3.867, −1.055]**
Wall following (proportion)	Gaussian	Identity	1 – y Box–Cox (λ = 0.1)	**(Intercept)**	**0.910**	**0.011**	**83.068**	**<0.001**	**[0.8868, 0.931]**
				Night	−0.001	0.003	−0.430	0.667	[−0.005, 0.004]
Shelter proximity (proportion)	Gaussian	Identity	Box–Cox (λ = 0.1)	**(Intercept)**	**−3.227**	**0.157**	**−20.564**	**<0.001**	**[−3.537, −2.918]**
				Night	0.091	0.053	1.725	0.083	[−0.014, 0.193]
				Shelter (LS)	0.323	0.205	1.571	0.114	[−0.081, 0.720]
				Night × shelter (LS)	**−0.187**	**0.074**	**−2.534**	**0.011**	**[−0.333, −0.041]**
Mean speed (mm s−1)	Gaussian	Log	–	**(Intercept)**	**2.053**	**0.164**	**12.513**	**<0.001**	**[1.731, 2.374]**
				Night	−0.082	0.051	−1.608	0.108	[−0.182, 0.018]
				Path type (out)	**0.518**	**0.210**	**2.462**	**0.014**	**[0.105, 0.930]**
				Night × path type (out)	**−0.144**	**0.072**	**−2.006**	**0.045**	**[−0.284, −0.003]**
Mean distance to wall (cm)	Gaussian	Log	–	**(Intercept)**	**−1.884**	**0.295**	**−6.397**	**<0.001**	**[−2.461, −1.306]**
				Night	0.019	0.082	0.228	0.820	[−0.141, 0.179]
				Path type (out)	**0.517**	**0.248**	**2.085**	**0.037**	**[0.031, 1.0036]**
				Night × path type (out)	−0.077	0.071	−1.082	0.279	[−0.216, 0.062]
Sinuosity (rad $\sqrt{m^{-1}}$)	Gamma	Log	–	**(Intercept)**	**1.349**	**0.197**	**6.837**	**<0.001**	**[0.962, 1.735]**
				Night	−0.047	0.042	−1.134	0.257	[−0.129, 0.034]
				Path type (Out)	**−0.377**	**0.181**	**−2.076**	**0.038**	**[−0.732, −0.021]**
				Night × path type (out)	0.040	0.052	0.775	0.439	[−0.062, 0.142]
Vector length	Gamma	Log	log $y/1-y$	**(Intercept)**	**1.948**	**0.305**	**6.381**	**<0.001**	**[0.978, 2.767]**
				Night	**−0.284**	**0.101**	**−2.809**	**0.005**	**[−0.577, −0.013]**

The assumed distributions of residuals are indicated by Family. Link functions and transformations of the response variable (if used) are indicated. Confidence intervals are calculated using semiparametric bootstrap resampling (n = 1000), and p values are calculated using normal approximation. Bold indicates significance (α = 0.05)

farthest two walls was 0.89 m, so the 0.84 m criterion ensured that a subject was at least 5 cm from the opposite walls at the start (inbound) or end (outbound) of the paths. Importantly, this criterion also controlled for the inflation of path sinuosity that would have occurred if an animal wandered back and forth on the opposite walls of the arena before it returned to the shelter.

Each outbound and inbound path was characterized by mean linear speed, mean distance to the wall, and sinuosity (Bovet and Benhamou 1988). We fitted a GLMM to each variable to test for changes in behavior across nights and to identify differences between the outbound and inbound paths. For each model, we included night number, path type, and the interaction between night number and path type as fixed effects.

Outbound and inbound route fidelity were evaluated from vector angles for the respective paths, calculated as a unit vector with the mean absolute angle for each trajectory from the start (inbound) or end (outbound) of the path to the HS entrance. To determine whether animals used a directionally similar inbound and outbound route each night, we calculated the mean vector for the outbound and inbound vectors for each subject within nights, and we fitted a GLMM to the resultant outbound–inbound vector length that included night number as a fixed effect.

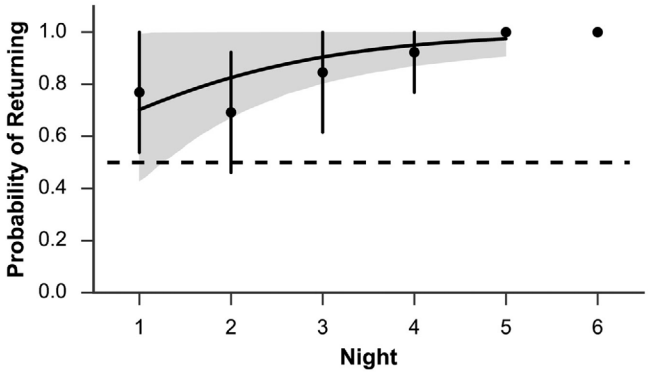


Fig. 2. Shelter fidelity. *Dots* show the mean probability (±95% CI) of returning to HS for each night, and the *solid line* (± 95% CI) shows the GLMM fitted to the data. The *dashed line* indicates the random expectation (0.50). The first five nights of data were used to fit the model (n = 12). Data from night 6 (n = 8) are shown only to demonstrate the robustness of shelter fidelity after the initial five-night session.

Finally, we visually inspected extended versions of all inbound paths for clues related to possible homing strategies. Plots along with qualitative descriptions of selected homing trajectories are provided.

Model selection

For shelter choice, we used a Bernoulli logit-link GLMM due to the binomial distribution of the response data. For all other variables, we began model selection by fitting a Gaussian identity-link GLMM, the residuals from which were assessed for normality, heteroscedasticity, and nonlinear patterns by inspecting a plot of the Pearson residuals by fitted values and a quantile–quantile normal probability plot of the residuals. Based on these results, we chose an appropriate error distribution and link function for each response variable until the distribution of the residuals was satisfactory and the model successfully converged. If an appropriate error distribution was not found or the model did not converge, the response variable was transformed, and we restarted the model selection process.

For cases where we transformed the response variable, the fixed effects coefficients are given in transformed space, but our visualizations are reverse transformed. All models were fitted with restricted maximum likelihood and reported p values for fixed effects are normal approximations.

Software

We performed all data processing and statistical analyses in Python 2.7.11 with the SciPy stack (Perez et al. 2011) and in R 3.3.0 (R Development Core Team 2016). For GLMM analyses, we used the R package lme4 1.1.12 (Bates et al. 2014). We calculated semiparametric bootstrapped (n = 1000) 95% confidence intervals (CIs) using confint.merMod from lme4. For Box–Cox and power transforms and their inverses, we used the bxcx function from the R package FitAR 1.94 (McLeod and Zhang 2008). Visualizations were created using the Python packages matplotlib 1.5.1 (Hunter 2007) and seaborn 0.7.1 (Waskom et al. 2016). The schematics in Fig. 1 were created with SketchUp 16.0 (Trimble Navigation Ltd.).

Results

The experimental session ended after five consecutive nights for ten subjects and after six nights for the two other subjects. Results for each GLMM, including error distributions and link functions, are given in Table 2. Variables in the text are written as mean ± standard error.

Site Fidelity

Phrynus marginemaculatus reliably homed and learned to discriminate between the two shelters. Initially, subjects utilized the two shelters equally, but the probability that the HS was used increased on each successive night and subjects reached perfect fidelity to the HS by the fifth night (Fig. 2). Eight subjects that completed the session in five consecutive nights were held in the arena for a sixth night and all of these individuals again utilized the HS. Half of the 12 subjects never utilized the LS and, hence, did not experience the poorer light environment of the LS.

Phenology and general activity

Individuals exhibited distinctive daytime and nighttime activity patterns (Fig. 3a). Daylight hours were spent in a shelter, and subjects spent, on average, 7.53 ± 0.87 h of the 12-h dark period outside the shelters. Subjects consistently exited their shelter each night on average 1.57 ± 0.35 h after all lights went out, and their exit time from a shelter did not change across nights (Fig. 3b). Subjects returned to their shelter on average 2.96 ± 0.63 h before the overhead lights came on, but returned earlier each night as the session progressed (Fig. 3c). Thus, the overall duration of nocturnal activity decreased over the session (Fig. 3d). This reduced activity period corresponded to a reduction in the distance travelled by subjects in the arena. Individuals moved an average distance of 169.97 ± 97.23 m on the first night, and this distance decreased to less than half this distance (83.02 ± 76.98 m) by the fifth night (Fig. 3e).

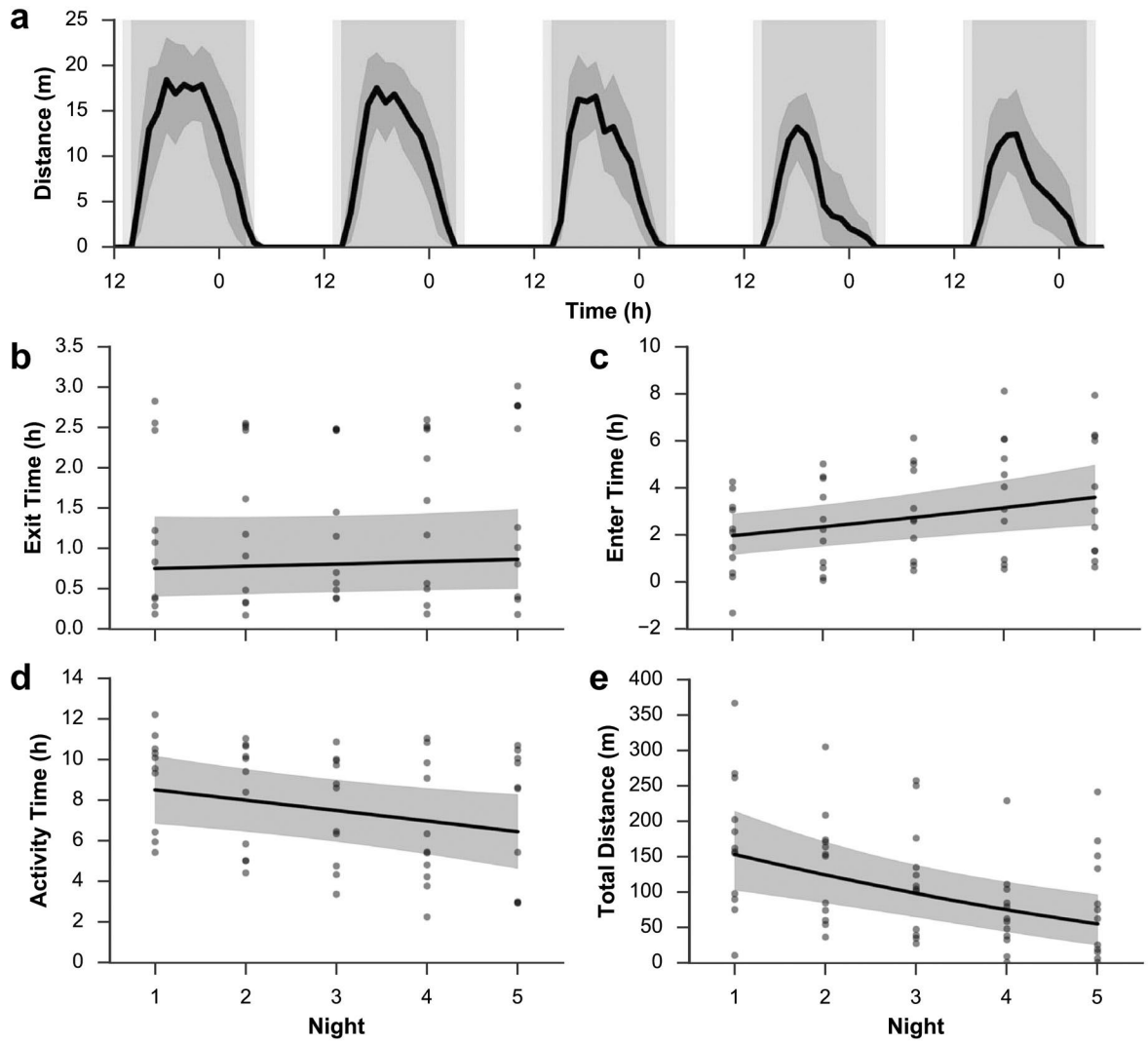


Fig. 3. Nocturnal phenology and activity in the arena. **a** Actogram illustrating general activity patterns across nights. *Dark gray bars* indicate the time intervals when the overhead lights and arena lights were turned off, while *light gray bars* indicate the time intervals when overhead lights were on and arena lights were off. *Black lines* show the mean (±95% CI) for distance moved, binned for each hour for all animals (n = 12). Activity is highest in the middle of the night with less activity toward the beginning and end of the night. **b–e)** Visualizations of the GLMMs fitted to the phenology variables. *Black lines* show the mean trend (±95% CI). On average, individuals exited the shelter at the same time each night, returned earlier across the session and, therefore, spent less time in the arena. The reduced activity period outside a shelter was associated with less total movement (distanced travelled).

Space utilization

The utilization of space in the arena across the session is shown in Fig. 4a. The proportion of steps for which a subject was within 5 cm of a wall was greater than the random expectation on all nights and did not decrease over the session (Fig. 4b). On average, more than half of the movement trajectory for each subject was in proximity to a wall. As the session progressed, the proportion of movement near the HS did not change across nights, while movement around the LS decreased to below chance level (Fig. 4c).

Outbound and inbound paths

Figure 5 shows all outbound and inbound trajectories used for analysis, where subjects both exited and re-entered the HS. Mean linear speed was on average greater for outbound paths, but unlike inbound paths, declined rapidly across nights (Fig. 5b). Mean distance to the wall was greater for outbound paths and did not change across nights for either outbound or inbound paths (Fig. 5c). Sinuosity was greater for inbound paths but also did not change across nights for either inbound or outbound paths (Fig. 5d).

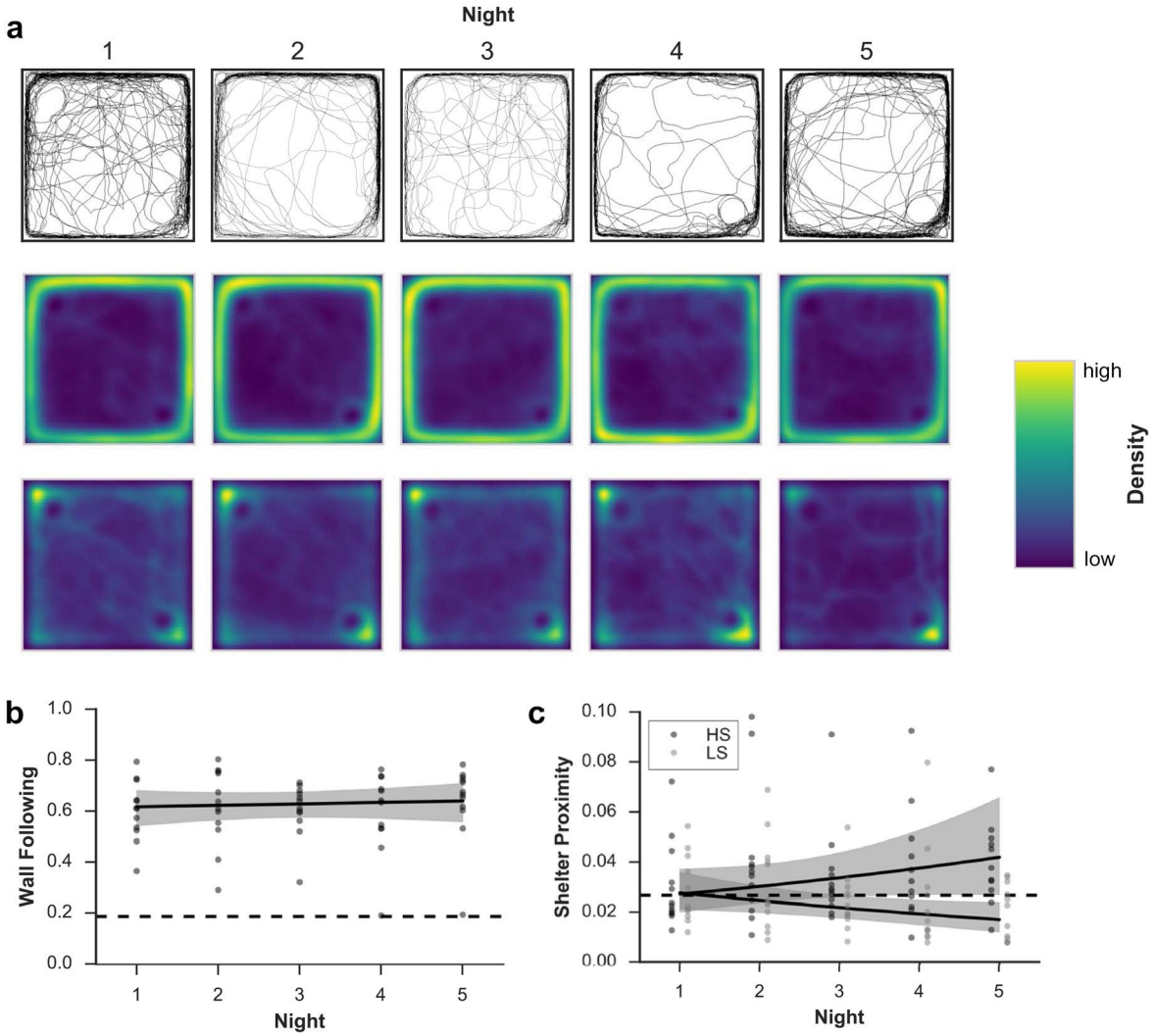


Fig. 4. Space use by subjects across nights. **a)** The full movement trajectory for one subject on each night (*top*) along with the mean 2D Gaussian kernel density estimate (KDE) of space use across nights for all subjects (*middle*) and the mean KDE for steps >5 cm from the wall (*bottom*). The LS is located in the *top-left*, while the HS is in the *bottom-right*. The *bottom* KDE plots illustrate the decrease in the proportion of movement in proximity to the LS as the session progressed. **b)** *Dots* show the mean proportion of steps recorded in the outer 5 cm of the arena. The *solid line* (± 95% CI) shows the estimated effect of time (nights) from the GLMM. The proportion of steps in the outer 5 cm of the arena was consistently higher than the random expectation (0.18; *dashed line*) but did not change across nights. **c)** *Dots* show the mean proportion of steps recorded around each shelter across nights. The corresponding *solid lines* (± 95% CI) show the estimated effect of time (nights) from the GLMM. The proportion of recorded steps near the HS and LS started at chance levels (*dashed line*) and diverged as the session progressed. The proportion of steps around the HS did not significantly change, while the proportion of steps near the LS decreased below chance.

Figure 6 shows the angular characteristics of outbound and inbound paths relative to the HS for all subjects. These data further illustrate the results found in the kinematic analyses, namely that inbound paths tended to be guided by the wall, with most mean vector angles near 0° and 90°. Additionally, we found that outbound–inbound vector length decreased across nights, which implies that individuals exhibited lower outbound–inbound route fidelity the longer they were in the arena (Fig. 6b).

The majority of inbound paths to the HS (44 of 50) can be characterized by three behavioral sequences (Fig. 7). These trajectories include situations in which a subject either (a) briefly sampled the area around the LS, walked along the wall of the arena, and then approached the HS from near a wall (Fig. 7a) (b) briefly sampled the area around the LS and then exhibited area-restricted search around the HS (Fig. 7b) or (c) first sampled the area near the LS petri dish and subsequently sampled the HS petri dish with the odor cue (Fig. 7c). Three subjects returned directly to the HS without conspicuous use of one of these strategies. These subjects sampled the area around the LS and then returned to the HS without any prolonged search

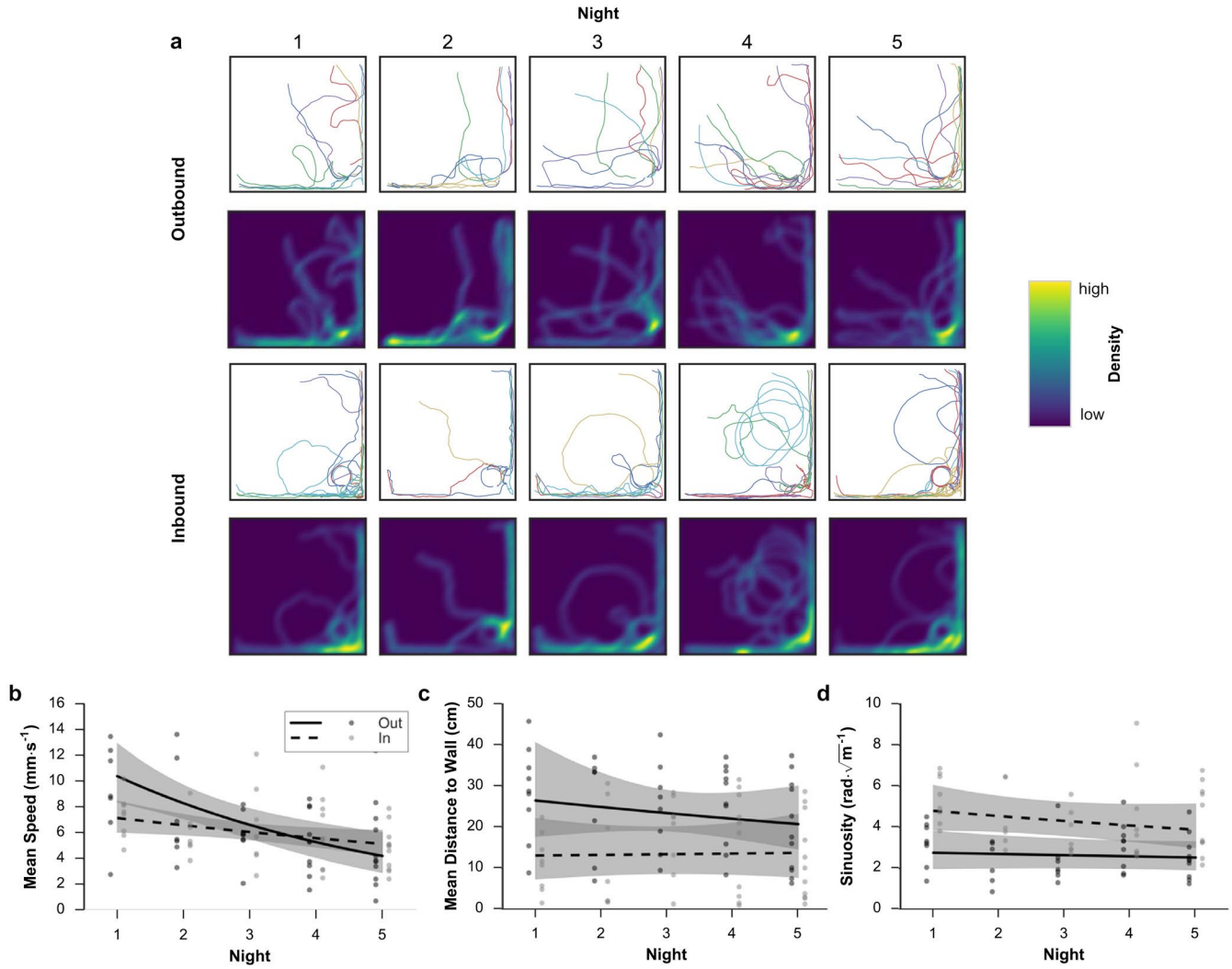


Fig. 5. Path kinematics. **a)** The outbound and inbound trajectories for all subjects used for the analysis and their 2D Gaussian kernel density estimates. Local density peaks near the walls and shelter indicate both consistent space use between subjects and back and forth movement within trajectories. The LS is located in the *top-left*, while the HS is in the *bottom-right*. **b** Mean linear speed is generally higher on outbound than inbound paths, although outbound speed declines and converges across nights. **b)** The mean distance to a wall was higher for inbound paths and did not change across nights. **c)** The sinuosity was greater for inbound paths and did not change across nights. *Dots* show the mean values for each path type across nights. The black lines (±95% CI) show the estimated effect of time (nights) from the GLMM.

behavior near the HS (Fig. 7d). Finally, a single subject exhibited a unique spiral-like search pattern on its return to the HS on three nights (Fig. 7e).

Discussion

The results of this study show that *P. marginemaculatus* reliably exit and return to a shelter at night in a laboratory and develop site fidelity when available shelters differ in quality. Preference for the HS increased rapidly over the session, demonstrating that subjects quickly learned to discriminate between the two shelters. The development of shelter fidelity as the session progressed was associated with decreased movement near the LS but not HS. In addition, the animals exited shelters consistently, but returned earlier and exhibited lower overall activity as the session progressed. The linear speed of outbound paths, unlike inbound paths, also decreased and outbound, and inbound paths directionally diverged. Together, these observations suggest that subjects developed a familiarity with the HS and exploration of the alternative refuge site decreased as the animal learned the lower quality of the alternative. Indeed, the reduction of overall activity is typical of many animals introduced to a novel environment (Teyke 1989; Mikheev and Andreev 1993). Across nights, the motivation to explore a once novel environment would necessarily decrease with the growing familiarity. The

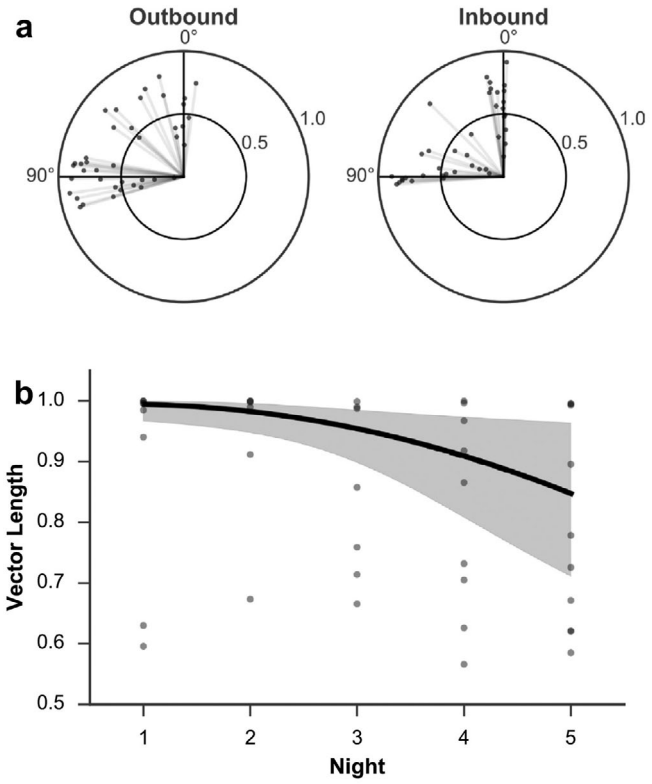


Fig. 6. Mean path vectors. **a)** The distribution of mean vectors for outbound and inbound paths relative to the HS. **b** GLMM showing the effect of night on outbound–inbound vector length. The *solid black line* is the average trend (±95% CI). Outbound–inbound vector length significantly decreased across nights.

reduction of time outside a shelter as the session progressed might also be explained, in part, by fatigue, as animals were not fed in the arena.

In this study, our goals were to determine whether *P. marginemaculatus* could be trained to exhibit shelter fidelity in the laboratory and to characterize changes in movement and space use associated with the development of fidelity to a shelter. No sensory cues were manipulated, but the design of the experiment placed constraints on the information that could be used. In particular, *P. marginemaculatus* must have relied on a sensory modality other than vision to orient and home successfully because subjects were forced to relocate a shelter in complete darkness. Their ability to home successfully in the dark is perhaps not unexpected, as vision has been assumed to rarely play an important role in nocturnal arachnid navigation (Foelix 1996). However, many nocturnal arthropods, including arachnids, rely on vision for nocturnal navigation (Dacke et al. 1999; Ortega-Escobar 2002, 2006, 2011; Nørgaard et al. 2007, 2008, 2012; Reyes-Alcubilla et al. 2009; Ortega-Escobar and Ruiz 2014; reviewed by; Warrant and Dacke 2010, 2011, 2016). In amblypygids, little is known about the role of visual information for navigation, but displacement studies on *P. pseudoparvulus* in Costa Rica suggest that navigation is at most moderately impeded when animals are vision deprived (Hebets et al. 2014b; Bingman et al. 2017).

Path integration is a common navigation mechanism in central-place foragers, but our analyses did not reveal the stereotypical differences in movement features between outbound and inbound trajectories relative to the location of a goal. These differences are well documented across taxa, including arachnids (Seyfarth and Barth 1972; Seyfarth et al. 1982), hymenopterans (Müller and Wehner 1988, 1994), decapods (Layne et al. 2003a, b; Kamran and Moore 2015), and mammals (Etienne et al. 1996; Etienne and Jeffery 2004; Wallace et al. 2006). In particular, animals walk slowly and their paths are more sinuous on outbound journeys while inbound paths are fast, straight, and highly directed along an integrated homing vector. In this study, outbound speed was greater than or equal to that of inbound paths on each night, and sinuosity was greater for inbound than for outbound paths, the opposite of a typical journey relying on path integration. Furthermore, inbound paths were consistently in close proximity to a wall, when following a wall was not generally the most direct route to the HS. These path-type differences cast doubt on the idea that path integration was used as a shelter re-location strategy, but it is feasible, of course, that path integration by amblypygids fails to follow these general patterns.

The only controlled spatial cue provided in our experimental arena was a chemical odor, which we inferred, based on field experiments, could provide useful information to relocate the HS (Bingman et al. 2017). Indeed, navigation performance in the field is severely impaired when the sensory sensilla at the distal tips of the antenniform legs—the exclusive location of olfactory sensilla—are made nonfunctional (Beck and Görke 1974; Hebets et al. 2014b; Bingman et al. 2017). Furthermore, amblypygids possess more olfactory glomeruli—structures dedicated to olfactory processing— than many previously studied arthropods, which implies that they can detect and discriminate amongst a large number of odors. The hypothesis that odors are essential for navigation by amblypygids is also supported by the fact that they possess enormous mushroom bodies, an invertebrate brain region associated with olfactory learning, spatial memory, and sensory integration (Strausfeld et al. 1998; Wolff and Strausfeld 2015). This brain region contains several million neurons, and variation in its size and complexity has been attributed to the use of olfactory maps and the intensity of navigational demands (Farris and Schulmeister 2011; Jacobs 2012; Strausfeld 2012; Wolff and Strausfeld 2015; but see; Pfeiffer and Homberg 2014; Turner-Evans and Jayaraman 2016).

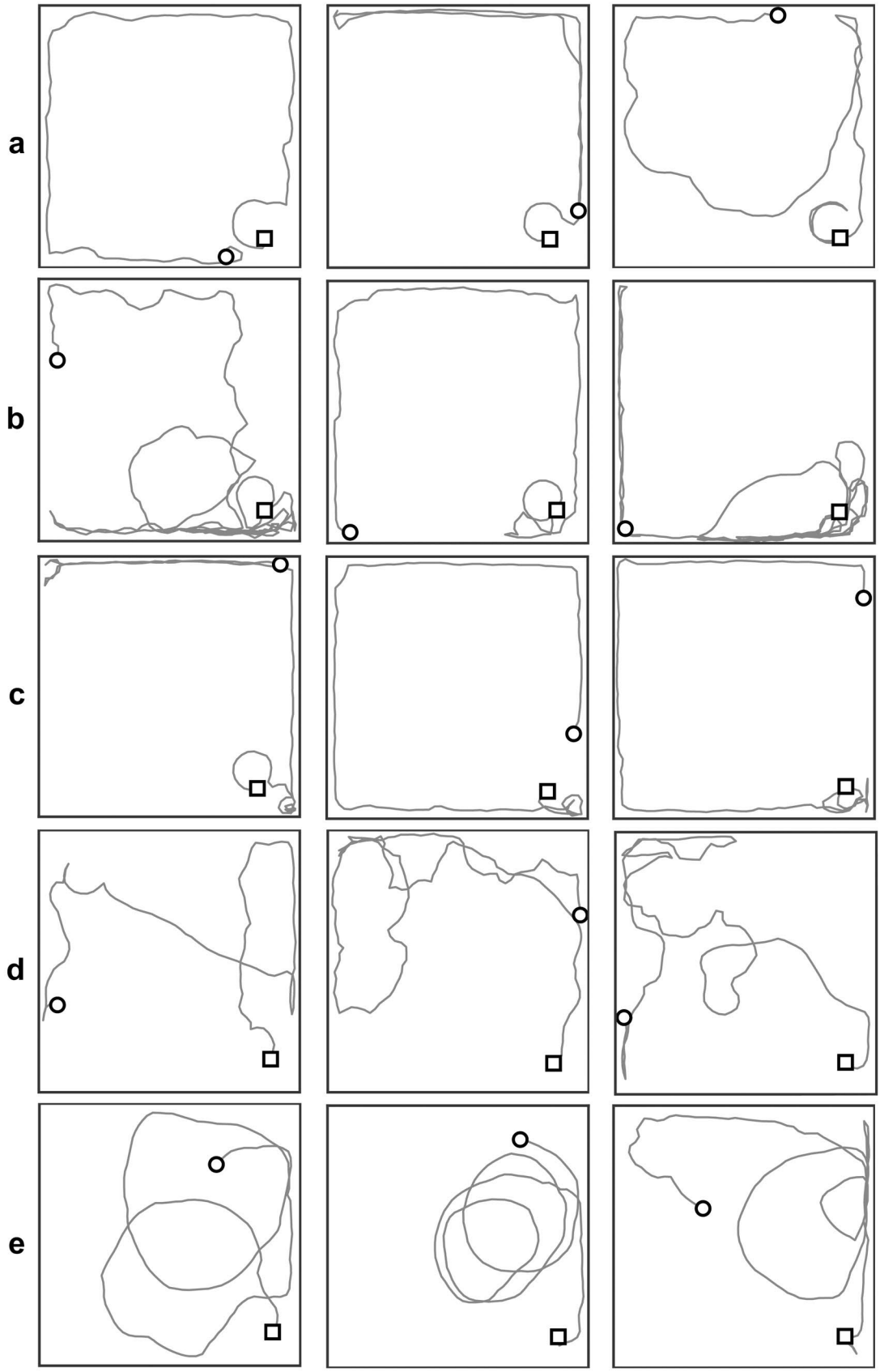


Fig. 7. Extended inbound paths. **a–c)** Representative paths that illustrate behavioral sequences evident in 44 of the 50 paths analyzed. **d, e)** Paths that were rarely exhibited. *Open circles* indicate the start of each path, while *open squares* indicate the end of each path.

Additional suggestions regarding the potential role of the odor source as a learned landmark come from comparisons of inbound and outbound trajectories (Fig. 5c) and extended observations of these paths (Fig. 7). Inbound paths were consistently closer to the walls of the arena than outbound paths, and persistent movement along the walls of the arena unavoidably led to the location of the odor cue near the HS. Furthermore, most extended trajectories showed what appears to be area-restricted search around the HS preceded by less intense search around the LS. Subjects in Fig. 7c, in particular, sampled the area around the petri dish laden with water and then walked to and more intensively sampled the dish laden with geraniol before they entered the HS. The subject in Fig. 7e was observed to move circuitously across the center of the arena and then enter the HS which is reminiscent of paths associated with the use of a beacon (Geva-Sagiv et al. 2015). This individual performed what appears to be a type of spiral search similar to olfactory-guided behavior observed in other animals (Vickers 2000; Calhoun et al. 2014; Svensson et al. 2014). The behavior of this subject, however, was not expressed by other individuals. Detailed observations of a larger sample of individuals and direct manipulation of odors could shed more light on our hypothesized olfactory-guided behavior.

Field studies of navigation behavior in complex environments, like a rainforest, have two notable impediments: the experimental manipulation of the sensory cues is difficult to implement, and detailed movements of individuals cannot be easily measured. Here, we established that *P. marginemaculatus* can be trained to home to an artificial shelter in the laboratory, and we documented changes in behavior associated with the development of site fidelity, which is often rapid. This system provides an opportunity to study the behavioral, sensory, and neural mechanisms hypothesized to control navigation under conditions in which sensory information can be manipulated and tightly controlled. Indeed, the detail with which movements can be quantified in the laboratory and the increasing availability of computational tools for analyzing these data should allow researchers to detect even subtle differences in behavior caused by cue manipulations (see Anderson and Perona 2014; Berman et al. 2014; Dell et al. 2014; Egnor and Branson 2016).

Acknowledgments — Joseph Bak-Coleman and Robert Huber provided code used to track animals and perform statistical analysis; Sheryl Coombs, Stephen Lang, and two reviewers provided helpful comments on the manuscript; Steve Queen constructed portions of the behavioral arenas; and Connie Santangelo provided assistance with the experiments. This work was supported in part by the National Science Foundation (IOS 1457304). JMG also received summer stipend support from the Center for Undergraduate Research and Scholarship at Bowling Green State University.

References

Anderson DJ, Perona P (2014) Toward a science of computational ethology. Neuron 84(1):18–31

Baird E, Dacke M (2016) Finding the gap: a brightness-based strategy for guidance in cluttered environments. Proc R Soc B 283(1828). doi:10.1098/rspb.2015.2988

Bates D, Maechler M, Bolker B, Walker S (2014) lme4: linear mixed-effects models using Eigen and S4. R package version 1.1.12. http://CRAN.R-project.org/package=lme4

Beck L, Görke K (1974) Tagesperiodik, Revierverhalten und Beutefang der Geißelspinne Admetus pumilio CL Koch im Freiland. Z Tierpsychol 35(2):173–186

Beck L, Foelix R, Gödeke E, Kaiser R (1977) Morphologie, Larvalentwicklung und Haarsensillen des Tastbeinpaares der Geielspinne Heterophrynus longicornis Butler (Arach., Amblypygi). Zoomorphologie 88(3):259–276

Berman GJ, Choi DM, Bialek W, Shaevitz JW (2014) Mapping the stereotyped behaviour of freely moving fruit flies. J R Soc Interface 11(99):20140672

Bingman VP, Graving JM, Hebets EA, Wiegmann DD (2017) Importance of the antenniform legs, but not vision, for homing by the neotropical whip spider, *Paraphrynus laevifrons*. J Exp Biol. doi:10.1242/jeb.149823

Bolker BM, Brooks ME, Clark CJ, Geange SW, Poulsen JR, Stevens MHH, White JSS (2009) Generalized linear mixed models: a practical guide for ecology and evolution. Trends Ecol Evol 24(3):127–135

Bovet P, Benhamou S (1988) Spatial analysis of animals' movements using a correlated random walk model. J Theor Biol 131(4):419–433

Calhoun, AJ, Chalasani SH, Sharpee TO (2014) Maximally informative foraging by *Caenorhabditis elegans*. eLife 3:e04220

Chapin KJ, Hebets EA (2016) The behavioral ecology of amblypygids. J Arachnol 44(1):1–14

Cheng K (2012) Arthropod navigation: ants, bees, crabs finding their way. In: Zentall TR, Wasserman E (eds) The Oxford handbook of comparative cognition. Oxford University Press, Oxford, pp 347–365

Cheng K, Freas CA (2015) Path integration, views, search, and matched filters: the contributions of Rüdiger Wehner to the study of orientation and navigation. J Comp Physiol A 201(6):517–532

Collett M, Collett TS (2006) Insect navigation: no map at the end of the trail? Curr Biol 16(2):R48–R51

Collett TS, Graham P (2004) Animal navigation: path integration, visual landmarks and cognitive maps. Curr Biol 14(12):R475–R477

Collett M, Chittka L, Collett TS (2013) Spatial memory in insect navigation. Curr Biol 23(17):R789–R800

Dacke M, Nilsson DE, Warrant EJ, Blest AD, Land MF, O'Carroll DC (1999) Built-in polarizers form part of a compass organ in spiders. Nature 401(6752):470–473

Dell AI, Bender JA, Branson K, Couzin ID, de Polavieja GG, Noldus LP, Brose U (2014) Automated image-based tracking and its application in ecology. Trends Ecol Evol 29(7):417–428

Dyer FC (1998) Cognitive ecology of navigation. In: Dukas R (ed) Cognitive ecology. University of Chicago Press, Chicago, pp 201–260

Egnor SR, Branson K (2016) Computational analysis of behavior. Annu Rev Neurosci (0)

Etienne AS, Jeffery KJ (2004) Path integration in mammals. Hippocampus 14(2):180–192

Etienne AS, Maurer R, Séguinot V (1996) Path integration in mammals and its interaction with visual landmarks. J Exp Biol 199(1):201–209

Farris SM, Schulmeister S (2011) Parasitoidism, not sociality, is associated with the evolution of elaborate mushroom bodies in the brains of hymenopteran insects. Proc R Soc B 278(1707):940–951

Foelix RF (1996) Biology of spiders, 2nd edn. Oxford University Press, Oxford

Foelix RF, Chu-Wang IW, Beck L (1975) Fine structure of tarsal sensory organs in the whip spider *Admetus pumilio* (Amblypygi, Arachnida). Tissue Cell 7(2):331–346

Geva-Sagiv M, Las L, Yovel Y, Ulanovsky N (2015) Spatial cognition in bats and rats: from sensory acquisition to multiscale maps and navigation. Nat Rev Neurosci 16(2):94–108

Gould JL (1998) Sensory bases of navigation. Curr Biol 8(20):R731–R738

Graving JM (2016) POSE: Python Open Source Ethology Tracker. Python package version 0.1.1-beta (September 2016). Zenodo. http://doi.org/10.5281/zenodo.61738

Hansson BS, Stensmyr MC (2011) Evolution of insect olfaction. Neuron 72(5):698–711

Harvey MS (2007) The smaller arachnid orders: diversity, descriptions and distributions from Linnaeus to the present (1758 to 2007). Zootaxa 1668(1):363–380

Hebets EA (2002) Relating the unique sensory system of amblypygids to the ecology and behavior of *Phrynus parvulus* from Costa Rica (Arachnida, Amblypygi). Can J Zool 80(2):286–295

Hebets EA, Chapman RF (2000) Electrophysiological studies of olfaction in the whip spider *Phrynus parvulus* (Arachnida, Amblypygi). J Insect Physiol 46(11):1441–1448

Hebets EA, Gering EJ, Bingman VP, Wiegmann DD (2014a) Nocturnal homing in the tropical amblypygid *Phrynus pseudoparvulus* (Class Arachnida, Order Amblypygi). Anim Cogn 17(4):1013–1018

Hebets EA, Aceves-Aparicio A, Aguilar-Argüello S, Bingman VP, Escalante I, Gering EJ, ... Stafstrom JA (2014b) Multimodal sensory reliance in the nocturnal homing of the amblypygid *Phrynus pseudoparvulus* (Class Arachnida, Order Amblypygi). Behav Process 108:123–130

Hunter JD (2007) Matplotlib: a 2D graphics environment. Comput Sci Eng 9(3):90–95

Jacobs LF (2012) From chemotaxis to the cognitive map: the function of olfaction. Proc Natl Acad Sci USA 109(Supplement 1):10693–10700

Jeffery KJ, Jovalekic A, Verriotis M, Hayman R (2013) Navigating in a three-dimensional world. Behav Brain Sci 36(05):523–543

Kamran M, Moore PA (2015) Comparative homing behaviors in two species of crayfish, *Fallicambarus fodiens* and *Orconectes rusticus*. Ethology 121(8):775–784

Knaden M, Graham P (2016) The sensory ecology of ant navigation: from natural environments to neural mechanisms. Annu Rev Entomol 61:63–76

Layne JE, Barnes WJ, Duncan LM (2003a) Mechanisms of homing in the fiddler crab *Uca rapax* 1. Spatial and temporal characteristics of a system of small-scale navigation. J Exp Biol 206(24):4413–4423

Layne JE, Barnes WJP, Duncan LM (2003b) Mechanisms of homing in the fiddler crab *Uca rapax* 2. Information sources and frame of reference for a path integration system. J Exp Biol 206(24):4425–4442

Leonard AS, Masek P (2014) Multisensory integration of colors and scents: insights from bees and flowers. J Comp Physiol A 200(6):463–474

McLeod AI, Zhang Y (2008) Improved subset autoregression: with R package. J Stat Softw 28(2):1–28

Mikheev VN, Andreev OA (1993) Two-phase exploration of a novel environment in the guppy, *Poecilia reticulata*. J Fish Biol 42(3):375–383

Müller M, Wehner R (1988) Path integration in desert ants, *Cataglyphis fortis*. Proc Natl Acad Sci USA 85(14):5287–5290

Müller M, Wehner R (1994) The hidden spiral: systematic search and path integration in desert ants, *Cataglyphis fortis*. J Comp Physiol A 175(5):525–530

Nørgaard T, Henschel JR, Wehner R (2007) Use of local cues in the night-time navigation of the wandering desert spider *Leucorchestris arenicola* (Araneae, Sparassidae). J Comp Physiol A 193(2):217–222

Nørgaard T, Nilsson DE, Henschel JR, Garm A, Wehner R (2008) Vision in the nocturnal wandering spider *Leucorchestris arenicola* (Araneae: Sparassidae). J Exp Biol 211(5):816–823

Nørgaard T, Gagnon YL, Warrant EJ (2012) Nocturnal homing: learning walks in a wandering spider? PloS One 7(11):e49263

Ortega-Escobar J (2002) Evidence that the wolf-spider *Lycosa tarentula* (Araneae, Lycosidae) needs visual input for path integration. J Arachnol 30(3):481–486

Ortega-Escobar J (2006) Role of the anterior lateral eyes of the wolf spider *Lycosa tarentula* (Araneae, Lycosidae) during path integration. J Arachnol 34(1):51–61

Ortega-Escobar J (2011) Anterior lateral eyes of *Lycosa tarantula* (Araneae: Lycosidae) are used during orientation to detect changes in the visual structure of the substratum. J Exp Biol 214(14):2375–2380

Ortega-Escobar J, Ruiz MA (2014) Visual odometry in the wolf spider *Lycosa tarantula* (Araneae: Lycosidae). J Exp Biol 217(3):395–401

Papi F (1992) Animal homing. Springer, The Netherlands

Patton P, Windsor S, Coombs S (2010) Active wall following by Mexican blind cavefish (*Astyanax mexicanus*). J Comp Physiol A 196(11):853–867

Perez F, Granger BE, Hunter JD (2011) Python: an ecosystem for scientific computing. Comput Sci Eng 13(2):13–21

Perry CJ, Barron AB, Cheng K (2013) Invertebrate learning and cognition: relating phenomena to neural substrate. Wiley Interdiscip Rev Cogn Sci 4(5):561–582

Pfeiffer K, Homberg U (2014) Organization and functional roles of the central complex in the insect brain. Annu Rev Entomol 59:165–184

Pulli K, Baksheev A, Kornyakov K, Eruhimov V (2012) Real-time computer vision with OpenCV. Commun ACM 55(6)

Quintero Jr. D (1981) The amblypygid genus *Phrynus* in the Americas (Amblypygi, Phrynidae). J Arachnol 9:117–166

R Development Core Team (2016) R: a language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. http://www.r-project.org

Reyes-Alcubilla C, Ruiz MA, Ortega-Escobar J (2009) Homing in the wolf spider *Lycosa tarantula* (Araneae, Lycosidae): the role of active locomotion and visual landmarks. Naturwissenschaften 96(4):485–494

Santer RD, Hebets EA (2009) Tactile learning by a whip spider, *Phrynus marginemaculatus* CL Koch (Arachnida, Amblypygi). J Comp Physiol A 195(4):393–399

Santer RD, Hebets EA (2011) The sensory and behavioural biology of whip spiders (Arachnida, Amblypygi). Adv Insect Physiol 41(1)

Seyfarth EA, Barth FG (1972) Compound slit sense organs on the spider leg: mechanoreceptors involved in kinesthetic orientation. J Comp Physiol 78(2):176–191

Seyfarth EA, Hergenröder R, Ebbes H, Barth FG (1982) Idiothetic orientation of a wandering spider: compensation of detours and estimates of goal distance. Behav Ecol Sociobiol 11(2):139–148

Strausfeld NJ (2012) Arthropod brains: evolution, functional elegance, and historical significance. Belknap Press of Harvard University Press, Cambridge

Strausfeld NJ, Hansen L, Li Y, Gomez RS, Ito K (1998) Evolution, discovery, and interpretations of arthropod mushroom bodies. Learn Mem 5(1):11–37

Svensson G, Strandh M, Löfstedt C (2014) Movements in the olfactory landscape. In: Hansson LA, Åkesson S, Akesson S (eds) Animal movement across scales. Oxford University Press, Oxford, pp 195–218

Teyke T (1989) Learning and remembering the environment in the blind cave fish *Anoptichthys jordani*. J Comp Physiol A 164(5):655–662

Turner-Evans DB, Jayaraman V (2016) The insect central complex. Curr Biol 26(11):R453–R457

United States Fish and Wildlife Service (1999) Pine Rocklands. Multi-species recovery plan for South Florida. United States Fish and Wildlife Service, Vero Beach, Florida. 3-161-3-194

Vickers NJ (2000) Mechanisms of animal navigation in odor plumes. Biol Bull 198(2):203–212

Wallace DG, Hamilton DA, Whishaw IQ (2006) Movement characteristics support a role for dead reckoning in organizing exploratory behavior. Anim Cogn 9(3):219–228

Warrant E, Dacke M (2010) Visual orientation and navigation in nocturnal arthropods. Brain Behav Evol 75(3):156–173

Warrant E, Dacke M (2011) Vision and visual navigation in nocturnal insects. Annu Rev Entomol 56:239–254

Warrant E, Dacke M (2016) Visual navigation in nocturnal *insects*. Physiology 31(3):182–192

Waskom M, et al (2016) Seaborn: v0.7.1 (June 2016). Zenodo. doi:10.5281/zenodo.54844

Weygoldt P (1977) Coexistence of two species of whip spiders (genus *Heterophrynus*) in the neotropical rain forest (Arachnida, Amblypygi). Oecologia 27(4):363–370

Weygoldt P (2000) Whip spiders (Chelicerata, Amblypygi). Apollo Books, Stenstrup

Wolff GH, Strausfeld NJ (2015) Genealogical correspondence of mushroom bodies across invertebrate phyla. Curr Biol 25(1):38–44